Aquaculture 479 (2017) 442–446

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Inbreeding effects on reproductive traits in a breeding population of Pacific white shrimp *Penaeus (Litopenaeus) vannamei*

Lidia de los Ríos-Pérez[a], Gabriel R. Campos-Montes[b], Alfonso Martínez-Ortega[d], Héctor Castillo-Juárez[c], Hugo H. Montaldo[a,*]

[a] *Departamento de Genética y Bioestadística, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, 04510 Coyoacán, Cd.Mx., Mexico*
[b] *Departamento de El Hombre y su Ambiente, Universidad Autónoma Metropolitana, Unidad Xochimilco, Calzada del Hueso 1100, 04960 Coyoacán, Cd.Mx., Mexico*
[c] *Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana, Unidad Xochimilco, Calzada del Hueso 1100, 04960 Coyoacán, Cd.Mx., Mexico*
[d] *Maricultura del Pacífico S.A. de C.V., Av. Dr. Carlos Canseco 5994 2° piso, Colonia El Cid, 82110 Mazatlán, Sinaloa, Mexico*

ARTICLE INFO

Keywords:
Inbreeding depression
Reproductive traits
Penaeus (Litopenaeus) vannamei
Genetic purging

ABSTRACT

The objective of this study was to investigate the effect of inbreeding on total number of eggs (TNE), fertilization rate (FR), hatching rate (HR), and number of nauplii (NN) in a nucleus breeding population of *Penaeus (Litopenaeus) vannamei.* An experiment was designed in order to generate inbred families that were the product of sibling mating in four successive generations. Inbreeding coefficients (F) estimated from the pedigree, varied between 0 and 60.4%. Number of spawnings evaluated and mean (SD) for the inbreeding level of the dam were 142 and 6.38 (10.87) % in 2010, 181 and 10.50 (13.76) % in 2011 and 166, 8.70 (12.15) % in 2012. Mean (SD) for the inbreeding level of the egg was 14.22 (18.50) % in 2011 and 8.08 (11.18) % in 2012. Data were analyzed within-year (generation) using linear model methodology. Estimated changes in FR per 10% increase in the inbreeding coefficient of the dam in years 2010, 2011 and 2012 were −26.0% ($P < 0.05$), −12.7% ($P < 0.10$) and −3.0% ($P > 0.10$), respectively, while for the inbreeding coefficient of the egg they were −17.0% ($P < 0.05$), −10.4% ($P < 0.10$) and −2.4% ($P > 0.10$), respectively. Estimated changes in HR per 10% increase in the inbreeding coefficient of the dam in years 2010, 2011 and 2012 were −24.5% ($P < 0.05$), −16.0% ($P < 0.05$) and −0.7% ($P > 0.10$), respectively, while for the inbreeding coefficient of the egg they were −15.4% ($P < 0.05$), −11.3% ($P < 0.05$) and 3.9% ($P > 0.10$), respectively. Estimated changes in NN per 10% increase in the inbreeding coefficient of the dam in years 2010, 2011 and 2012 were −24.6% ($P < 0.05$), −15.1% ($P < 0.05$) and −2.9% ($P > 0.10$), respectively, while for the inbreeding coefficient of the egg were −19.3% ($P < 0.05$), −9.3% ($P < 0.10$) and 0.04% ($P > 0.10$), respectively. Effect of the F of the dam on TNE was not significant ($P > 0.10$) in any year. Effects of inbreeding depression decreased across generations for most traits, which may be an indication of purging effects associated with selection against lethal or detrimental recessive alleles. High levels of inbreeding may involve a decline in reproduction traits that must be accurately estimated and taken into consideration when designing breeding programs in *P. vannamei*.

1. Introduction

Populations undergoing selection have a finite number of individuals available to become broodstock and produce progeny, which leads to a reduction in effective population sizes after several generations. This reduction increases the probability of mating between relatives in the population, even under controlled mating systems, making a certain degree of inbreeding and loss of genetic variability over time unavoidable (Kincaid, 1983; Falconer and Mackay, 1996; Ponzoni et al., 2010).

In livestock species and laboratory animals, inbreeding often affects traits associated with reproductive ability and physiological efficiency, leading to a reduction in the mean phenotypic value of certain traits, a phenomenon known as inbreeding depression (Falconer and Mackay, 1996; Lynch and Walsh, 1998). The magnitude and practical consequences of inbreeding are variable because they depend on the trait, the genetic constitution of the species or populations within species, and on how these inbreeding effects interact with the environment (Hedrick and Kalinowski, 2000). Control of the inbreeding rate is important in selection programs because the higher the level of

* Corresponding author.
E-mail address: montaldo@unam.mx (H.H. Montaldo).

http://dx.doi.org/10.1016/j.aquaculture.2017.06.013
Received 3 February 2017; Received in revised form 7 June 2017; Accepted 11 June 2017
Available online 13 June 2017
0044-8486/

inbreeding, the higher the probability that inbreeding depression will occur (Falconer and Mackay, 1996).

Research about inbreeding effects for reproductive traits in shrimp is scarce, despite its economic importance (ranking second in terms of economic value for aquaculture production, exceeded only by salmon; FAO, 2016) and there is only one study in *Penaeus (Litopenaeus) vannamei* where Moss et al. (2008) in an experiment conducted over two years with data from two successive generations of inbred and outbred families found a significant effect of inbreeding on hatching rate of $-12.3 \pm 10.1\%$ per 10% increase in inbreeding coefficients with inbreeding levels of 0.0, 25 and 37.5%. However, inbreeding depression studies for reproductive traits are also scarce in other aquaculture species including crustaceans. Studies available involve rainbow trout (*Oncorhynchus mykiss*) (Gjerde et al., 1983; Su et al., 1996), channel catfish (*Ictalurus punctatus*) (Bondari and Dunham, 1987) and Coho salmon (*Oncorhynchus kisutch*) (Gallardo et al., 2004).

Although inbreeding has been shown to depress fitness traits in a number of aquaculture species, little is known about the effects of inbreeding on reproductive traits in shrimp, including *P. vannamei*. The objective of this study was to investigate the effect of inbreeding on number of eggs, fertilization rate, hatching rate and number of nauplii in a nucleus breeding population of *P. vannamei*.

2. Material and methods

The study was conducted in the Mexican hatchery Maricultura del Pacífico on the northwest coast of Mexico over the years 2010 to 2012 with three successive generations of shrimp families. The experiment was designed in order to generate inbred families by sibling mating in successive generations, as well as to yield some families with different inbreeding coefficients, including families with relatively low inbreeding levels. Mating between siblings and cousins was performed to obtain families with incremental levels of inbreeding. Inbreeding coefficients (F) were calculated using pedigree information, which comprises 11 generations (2002 – 2012), and were estimated using Pedigree Viewer software, Version 6.5f (Kinghorn, 2011). Inbreeding levels for the three years studied are displayed in Table 2.

Shrimp used in this experiment came from a breeding line developed in 2007 with the original aim of conserving genetic variability. In this line, selection was primarily performed within families and therefore the increase in inbreeding level across the years was low. This line was derived from an original selected line formed in 1998 from wild shrimp from the Mexican Pacific Ocean and aquaculture shrimp from Colombia, Ecuador, Venezuela, and the United States. From 1998 to 2002, selection was based on (1) full-sib family phenotypic means of body weight at harvesting around 130 days of age, and (2) within-family selection for weight at 130 days of age. From 2003 to 2007, the selection criteria were family breeding values obtained using mixed linear models and Best Linear Unbiased Prediction and within-family information for body weight at 130 days of age.

In 2007, this conservation line incorporated about 7% of non-related families from other populations. From 2008 onwards, a selection index that considered body weight at 130 days of age and survival from 65 to 130 days of age with relative weights 2:1 was considered. Approximately the top 95% of the families were selected from each generation. Within-family selection was also performed based on approximate individual size at two stages (approximately 85 and 100 days) and individual body weight at 145 days of age, keeping approximately 20% of the heaviest shrimp at the last stage. It is important to mention that within-family selection procedure is carried out on a second group of full-sibs kept in separated family cages in the genetic nucleus of the hatchery. Therefore, selected broodstock are full-sibs of those evaluated in the ponds at 130 days of age. In this population (one spermatophore per female) and until year 2010, mating was performed so that the expected inbreeding coefficient of the progeny did not exceed 3.25%. During the course of the experiment, the only artificial selection made was of female broodstock for increased reproduction rate by discarding progeny of females with a low number of nauplii.

2.1. Broodstock management and family production

For the production of the families, mature broodstock selected in each production cycle were individually tagged using numbered rings placed in one ocular peduncle and stocked into maturation tanks at a density of 23 shrimp/m^3. Maturation tank dimensions were 12×3 m with a water column of 0.35 m, kept at 28–29 °C with a salinity of 34 g/L and daily water exchange rate of 400%. Broodstock diet was based on commercial pellets containing 35 to 40% protein and fresh food consisting of a mixture of squid, mussel, polychaetes, crustaceans of the genus *Euphausia*, paprika and vitamin C to promote gonadal development. Mature and ready-to-spawn female breeders were artificially inseminated using 1:1 sire:dam mating ratio. Inseminated females were placed in individual 200 L spawning tanks, where they remained for a period of 6 h for spawning and were then placed back into maturation tanks. Eggs were collected in 15 L tanks, washed with 96 g/L iodine, and placed back into another 15 L tank with continuous aeration. Spawnings were produced in 5 days in 2010, 8 days in 2011 and 3 days in 2012.

2.2. Female reproductive traits

The effects of inbreeding were evaluated in four female reproductive traits, which are described below.

2.2.1. Fertilization rate (FR)

A total of 200 spawnings produced from 184 sires and 193 dams were evaluated for FR. Six hours after insemination, a 50-mL sample of the water where the spawning was kept was collected in a glass beaker. Eggs were collected at the center and bottom of the glass beaker through centripetal force, to then be collected with a Pasteur pipette. A 0.2×5 cm area was delimited in a microscope slide to count fertile eggs, counting from right to left on the same line in a compound microscope with a $10\times$ objective. We counted as fertilized eggs those showing outer and inner membrane that were in morula stage (Fig. 1). The number of total and fertilized eggs was determined as the average of the counts made by two observers on the same microscope slide. FR per spawning was calculated as $100 \times$ total number of fertilized eggs/total number of eggs. Averages for total number of eggs evaluated per female for FR were 87.2, 126.3 and 127.0 in 2010, 2011 and 2012, respectively.

2.2.2. Number of nauplii (NN), total number of eggs (TNE) and hatching rate (HR)

Between 16 and 18 h after insemination, NN and unhatched eggs per female was estimated using the sum of the counts of three independent samples of 1 mL taken with a pipette directly from the 15 L tank where the spawning was concentrated. Samples were taken by placing the pipette perpendicular to the water surface, while air stones were used to facilitate the suspension and homogenization of the spawning. TNE per dam was calculated as the sum of the unhatched eggs and the number of nauplii. HR per spawning was calculated as $100 \times$ number of nauplii/total number of eggs. Averages for total number of eggs evaluated per female for HR were 36.11, 22.8 and 38.7 in 2010, 2011 and 2012, respectively. Total 489 spawnings produced from 443 sires and 467 dams were evaluated for TNE and NN, while 459 spawnings produced from 422 sires and 444 dams were evaluated for HR.

To compare our numbers with those from other studies, total number of eggs and nauplii per female per spawning in a 15 L tank, can be obtained by multiplying the variables actually measured (TNE, NN) by 5000.